

Appl. No. 09/922,604
Amendment dated September 24, 2004
Reply to Office Action mailed April 7, 2004

Amendments to the Specification:

Please amend the paragraphs appearing at page 9, lines 3-20 as follows:

Wang resin having 9-fluorenylmethyloxycarbonyl-protected alanine (Ala-Fmoc) attached thereto (3 grams (g), 2.52 mmol, 1 equivalent, NovaBiochem™, La Jolla, CA) was transferred to a clean round-bottom, 100 mL flask, and a solution of piperidine (12 mL) in dimethylformamide (DMF; 18 mL) was added to the resin in the flask. The solution was swirled for 1 hour, and the resin was isolated in a sintered glass funnel. The resin was washed with DMF (3 x 30 mL) followed by dichloromethane (DCM; 3 x 30 mL) and allowed to dry under vacuum for 5 minutes. Preferred protecting groups are 9-fluorenylmethyloxycarbonyl (Fmoc) and t-butoxycarbonyl (Boc).

The partially-dried resin was transferred into a clean round-bottom, 100 mL flask, and DMF (10 mL) was added. Then, Boc-Asp(OBz)OH (3.25 g, 10.07 mmol, 4 equivalents; Aldrich™) was added, followed by diisopropylamine (2.83 mL, 2.04 g, 20.19 mmol, 8 equivalents) and 2-(1H-benzotriazole-1-yl)-1,2,3,3-tetramethyluroniumtetrafluoroborate (TBTU; 3.24 g, 10.09 mmol, 4 equivalents, Acros, Loughborough Leicestershire, UK). The slurry was allowed to react under anaerobic conditions over 12 hours. At the end of this time, the resin showed a negative ninhydrin test, indicating the completion of the coupling reaction. The resin was vacuum filtered and washed with DMF (3 x 30 mL) followed by DCM (3 x 30 mL). The resin was allowed to dry at room temperature under vacuum for 10 minutes before transferring it into a clean round-bottom, 100 mL flask.

Please amend the paragraph appearing at page 10, lines 12-19 as follows:

This compound (400 mg) was dissolved in methanol (250 mL), and palladium on carbon catalyst (Pd/C; 10%, 0.4g; Fluka™ Chemicals, Dorset UK) was added carefully. The flask was purged with hydrogen and kept at a positive hydrogen pressure. The solution was kept in this atmosphere for at least 4 hours. The catalyst was removed with a filtering aid (celite) and washed with methanol. The methanol washings were combined, and the solvent was removed (yield 200 mg). Mass spectrometer and NMR analysis showed that the free acid Asp-Ala diketopiperazine

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(3-methyl-2,5- diketopiperazine-6-acetic acid, 5) had formed without any cross contamination.

Please amend the paragraph appearing at page 11, line 8 through page 12, line 6 as follows:

To a solution of 3-methyl-2,5- diketopiperazine-6-acetic acid (0.151 g, 0.81 mmol, 1 equivalent, preparation described in Example 1, 5) in DMF (2.5 mL) was added carbonyl diimidazole (0.26 g, 1.60 mmol, 2 equivalents, Aldrich™ Chemical Co., Milwaukee, WI). After stirring at room temperature for 1 hour, solid ammonium acetate (0.63 g, 8.17 mmol, 10 equivalents, Aldrich™) was added. Stirring at room temperature was continued overnight, at which time the reaction was partitioned between water (20 mL) and ethyl acetate (10 mL). The aqueous layer was washed with a second aliquot of ethyl acetate (10 mL), and it was then evaporated to dryness under reduced pressure (61°C). Traces of DMF were removed by further co-evaporations with water and then toluene to give a white solid (362 mg). This was taken up into a minimum volume of methanol in DCM (20:80 v/v) and flushed through a silica plug (10 g, chromatography grade silica; Aldrich) using methanol in DCM (20:80 v/v). The solvent eluted was fractionated, and the appropriate fractions were pooled and evaporated under reduced pressure (40°C) to give a white solid. The product was then recrystallized from methanol to give the desired product (0.116 g, 76% yield, 6).

Please amend the paragraphs appearing at page 13, lines 13-28 as follows:

Wang resin having Ala-Fmoc attached thereto (3 g, 2.52 mmol, 1 equivalent, NovaBiochem™) was transferred to a clean round-bottom, 100 mL flask, and a solution of piperidine (12 mL) in DMF (18 mL) was added to the resin in the flask. The solution was swirled for 1 hour, and the resin was isolated in a sintered glass funnel. The resin was washed with DMF (3 x 30 mL) followed by DCM (3 x 30 mL) and allowed to dry under vacuum for 5 minutes. Preferred protecting groups are 9-fluorenylmethyloxycarbonyl (Fmoc) and t-butoxycarbonyl (Boc).

The partially dried resin was transferred into a clean round-bottom 100 ml flask and DMF (10 mL) was added. Then Boc-Glu(OBz)OH (3.40 g, 10.08 mmol, 4 equivalents, Sigma™, St.

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Louis, MO) was added, followed by diisopropylamine (2.83 mL, 2.04 g, 20.19 mmol, 8 equivalents, Aldrich) and TBTU (3.24 g, 10.09 mmol, 4 equivalents, Acros). The slurry was allowed to react under anaerobic conditions for 10 hours. At the end of this time, the resin showed a negative ninhydrin test, indicating the completion of the coupling reaction. The resin was vacuum filtered and washed with DMF (3 x 30 mL) followed by DCM (3 x 30 mL). The resin was allowed to air dry at room temperature under vacuum for 10 minutes before transferring it into a clean round-bottom 100 mL flask.

Please amend the paragraph appearing at page 15, lines 6-18 as follows:

To a solution of 3-methyl-2,5-diketopiperazine-7-acetic acid (0.153 g, 0.75 mmol, 1 equivalent, preparation described in Example 4, 9) in DMF (2.5 ml) was added carbonyl diimidazole (0.24 g, 1.48 mmol, 2 equivalents, AldrichTM). After stirring at room temperature for 1 hour, solid ammonium acetate (0.58 g, 7.52 mmol, 10 equivalents, Aldrich) was added. Stirring at room temperature was continued overnight, at which time the reaction was partitioned between water (20 ml) and ethyl acetate (10 ml). The aqueous layer was washed with a second aliquot of ethyl acetate (10 ml), and it was then evaporated to dryness under reduced pressure (56°C). Traces of DMF were removed by further co-evaporations with water and then toluene to give a white solid (317 mg). This was taken up into a minimum volume of methanol in DCM (20:80 v/v) and flushed through a silica plug using methanol in DCM (20:80 v/v). The solvent eluted was fractionated, and the appropriate fractions were pooled and evaporated under reduced pressure (40°C) to give a white solid. The product was then recrystallized from methanol to give the desired product **10** (0.086 g, 57%).